



## EMBEDDING MOLDS – A REVIEW AND PROPOSED CLASSIFICATION

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### ABSTRACT

Embedding is the process in which the tissues or the specimens are enclosed in a mass of the embedding medium using different types of mould e.g steel molds, glass mold, plastic molds etc. Embedding is the crucial step in determining the orientation of sectioning. The tissue blocks are very thin in thickness they need a supporting medium in which the tissue blocks are embedded. This supporting medium is called embedding medium. Various embedding substances are paraffin wax, celloidin, synthetic resins, gelatine, etc.

**Histopathology** (compound of three Greek words: *histos* "tissue, *pathos* "suffering", and *-logia* "study of") refers to the microscopic examination of tissue to study the manifestations of disease (Culling *et al.*, 1985). Specifically, in clinical medicine, histopathology refers to the examination of a biopsy or surgical specimen by a pathologist after the specimen has been processed and histological sections have been placed onto glass slides. In contrast, cytopathology examines free cells or tissue micro-fragments (Slaouid and Fiette, 2011)

In order to study tissues with a microscope they must be preserved (fixed) and cut into sections thin enough to be translucent. The process of fixation is done. Fundamentally it consists of a chemical or physical method of killing the tissue and yet retaining characteristic peculiarities of shape and structure. Following fixation, blocks of tissue must be cut into thin sections. One way is to make a firm block by freezing fresh or fixed tissue. Other techniques involve dehydration in alcohols and infiltration with paraffin, or some similar agent - a process called embedding.

Embedding is the process in which the tissues or the specimens are enclosed in a mass of the embedding medium using a mould. Since the tissue blocks are very thin in thickness they need a supporting medium in which the tissue blocks are embedded. This supporting medium is called embedding medium. Various embedding substances are paraffin wax, celloidin, synthetic resins, gelatine, etc (Culling CFA *et al.*, 1985).

This embedding is done in different types of molds. Sections 3 to 10 microns (3 to 10 thousandths of a millimeter) in thickness are cut on steel knives mounted in an instrument called a microtome, which has a precise mechanical advance (Bracegirdl 1978).

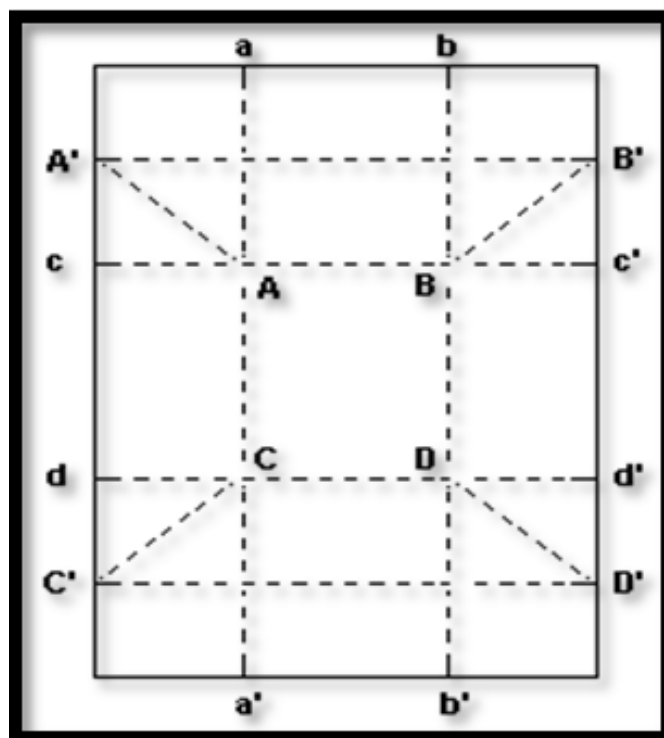
The present review reviews about the different embedding techniques and the molds used in the embedding of different tissues (Baker & Silverton's 1958)

### TYPES OF MOLDS:

1. Paper boat method
2. Ice tray methods
3. L-mold method
4. Plastic molds
5. Plastic embedding ring
6. Disposable molds
7. Steel molds

#### 1. Paper boat method:

Paraffin wax embedding is one of the oldest and widely used methods in the micro-technique studies. This has the advantage of being cheap to make and allowing block to be stored without being removed. This method is very useful for different cellular pathological as well as modern histological studies (Baker, Kumar S.R *et al.*, 2014). In this using a thick paper or thin card of suitable dimension. Fold it along the lines a'a' and b'b', then along c'c' and d'd', taking care to fold always the same way. (Culling CFA *et al.*, 1985)



**FIG 1:** Then make the folds AA', BB', CC', DD', still folding the same way. To do this you apply A c against A a, and pinch out the line AA', and so on for the remaining angles. This done, you have an imperfect tray with dogs' ears at the angles. To finish it, turn the dogs' ears round against the ends of the box, turn down outside the projecting flaps that remain, and pinch them down."

#### 2. Ice tray methods:

This method is used for hard embedding with the embedding medium resins (Caropreso, I *et al.*, 2000), wax etc. Carbowax, at 42 C, is poured into polyethylene ice cube trays, and the infiltrated blocks of tissue are pressed quickly to the bottoms of the individual compartments. The trays are put into a refrigerator (4 C.) until the Carbowax hardens. Blocks are then removed from the trays, placed on wooden or fiber pivots, and returned to the refrigerator until the entire thickness of the blocks is completely chilled. Thorough chilling is essential for obtaining good sections and will be hastened by trimming the faces of the blocks down to the tissue on a microtome before returning them to the refrigerator. The embedding mixture should be composed of equal parts of Carbowax 1000 and Carbowax 4000. If the temperature and humidity of the laboratory increase, the proportion of Carbowax 4000 (which contains no water) can be increased, and a firmer consistency of the blocks will thus be obtained. (Rose M *et al.*, 2000)

#### 3. Leuckhard mold:

A variety of moulds are used for embedding. Most of the laboratories use Leuckhard moulds. L moulds are made up of metal, easy to procure, reusable and may be adjusted to make different size of blocks. One limb of the "L" is longer than the other. The two "Ls" are jointed to form a sides of the rectangular box that act as a cast to make the mould. These are available in various sizes. (Culling

CFA 1985)



FIG 2: L -mold of various sizes

#### 4. Plastic molds:

These are relatively inexpensive, convenient and support the block during sectioning and are designed to fit it on the microtome. These are the disposable product. Are available in number of sizes and intended to speed up the paraffin wax embedding techniques. The plastic ring is used in conjunction with special stainless steel base moulds (FIG: 5). The tissue is embedded in position in a base and the plastic ring is placed in position and the paraffin wax poured in until it reaches the top. This eliminates the step of mounting or attaching the block on a holder (metal or wooden holder). Compound embedding units consist of square shaped brass or metal plates in a series of interlocking plates.

#### 5. Plastic embedding ring:

In this system plastic embedding rings with stainless steel (FIG: 5) moulds allow rapid embedding and cutting of tissues. In this system the blocks are stored with the plastic rings; the angle does not change for further requirement of sections. The disadvantage of this method is that the space required for storing is more (Baker and Silverton, 1998)



Fig 3: Plastic Embedding Rings

#### 6. Disposable molds:

In these types of molds, a sheet of cellulose acetate about 0.01 inches thick is clamped over a mold, heated to softness by an electric heater and drawn down over the mold by means of a vacuum. When cooled, the sheet, now formed into embedding boxes, is removed from the clamp. Boxes so made are inexpensive enough to be disposable but can be reused, since the sides of the boxes are sloped to allow easy removal of the paraffin block. (Joram P 1958)



Fig 4: Disposable molds

#### 7. Steel molds:

It provides a cassette to hold tissue during processing and has a stainless steel lid on the plastic cassette. The cassette has a rough surface on one side of it with a slope where the accession number or the marking is done using a permanent marker (Yuehwei H. et al., 2003) the main advantage is it is reusable



Fig: 5 Stainless Steel Moulds

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